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Overview of Inhalation Toxicology

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The development of inhalation toxicology as a distinct discipline can be traced back well over one hundred years. The technology has advanced in terms of materials and designs used to construct inhalation chambers and the equipment used to generate controlled test atmospheres of a wide variety of gases, vapors, dusts, and droplets. Consideration of metered dose inhalers, a relatively recent concern, has led to the design of new equipment for administering this unique dosage form. The parameters used to evaluate inhalation toxicity are similar to those used for any other route of administration. In addition, there are some unique procedures for early screening of pulmonary toxicity, especially within a series of related chemicals.

Introduction

The development of inhalation toxicology as a distinct discipline can be traced back well over a century. Fraser et al. (*1*) provided a brief review of this early period and a description of inhalation technology up to 1959. Inhalation toxicology technology has experienced continuous development in the types of materials and designs used in constructing inhalation chambers. Excellent reviews of inhalation technology have been provided by Campbell (*2*), Phalen (*3*), Drew (*4*), and MacFarland (*5*).

The following article will present an overview of aspects of current technology applied to inhalation toxicology studies, without attempting to review the entire spectrum of these studies. Some emphasis will be placed on studies with metered dose inhaler (MDI) aerosols, either for intranasal or pulmonary administration, as these represent an area of interest in drug delivery system development. Study design and inhalation toxicity assessment will be briefly considered.

Inhalation Toxicology Technology

Five basic types of inhalation toxicology studies have been described: whole body, head only, nose only, lung only, and partial lung (*6*). Each exposure type has its own unique set of advantages and disadvantages.

The advantages of whole-body inhalation systems include the ability to expose large numbers of animals simultaneously, accommodate a wide variety of species, employ minimal restraint, and suitability for chronic inhalation exposures. The major disadvantages include the large quantity of test material required for conduct of studies, multiple routes of exposure, distribution of concentration and particle size within the chamber exposure zone, control of chamber environment, and cost.

Head only and nose only inhalation exposure systems are similar enough to share advantages and disadvantages. The advantages include relative efficiency in usage of test material, reduction or elimination of multiple exposure routes, and containment of highly toxic materials. The major disadvantages are the labor intensive nature of head/nose only exposure systems, adequate exposure seals about the face or neck of animal models, and stress related to the restraint necessary for head/nose-only exposure studies.

Stress has, until recently, been a major limiting factor in determining the duration of head/nose only exposure studies (*4*). The need to restrain animals in tubes has been assumed to produce an undesirable level of stress when prolonged exposure is required. Smith et al. (*7*) have reported that long-term nose only exposures, up to 7 hr per day, were possible with little or no stress. Parameters such as body weight, rectal temperature, clinical pathology, and plasma corticosterone levels were used to indicate the lack of measurable stress in rats and hamsters for prolonged exposure periods. Laper and Burgess (*8*), however, have reported an increase in the acute inhalation toxicity related to restraint-induced stress.

Lung only and partial lung exposure techniques are also similar enough to share advantages and disadvantages. The major advantages are limited routes of exposure, direct knowledge of the actual quantity of test material delivered to the lung, and uniform delivery of multiple doses. Using other modes of inhalation exposure, i.e., whole body, the dose received during an inhalation exposure is a complex relationship between physiologic (rate, depth, and volume), and physical (particle size, collection efficiency, and retention) parameters. Disadvantages of the limited lung exposure techniques include the need for anesthesia and physiologic

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support, bypassing the nose, technical difficulty, limited number of animals that can be studied at any one time, and distribution of dose within the lung. Instillation of solutions or suspensions results in heavy, centralized deposits of material. Inhalation results in a lighter, even and widely distributed dose (*9*). While lung only or partial lung dosing procedures do not lend themselves to large scale screening/testing programs, they do have application in studies of absorption, metabolism, distribution, and excretion.

The design and construction of inhalation chambers have been extensively reviewed (*3–5,10,11*). Glass and various plastics have been used to construct inhalation chambers. Plastics, in general, tend to age rapidly with use. Both glass and plastic can build areas of high static charge, an undesirable feature in inhalation studies. Stainless steel is a common, and satisfactory material to use in inhalation chamber construction (Fig. 1). Glass windows are provided for observation. The poor thermal insulation properties of steel can be integrated into a chamber environmental control system (discussed later).

Inhalation exposure systems can be either static (no airflow) or dynamic (airflow). Static inhalation exposure systems, where a defined quantity of test material is introduced into a closed system and allowed to mix with the trapped air, are efficient in terms of test chemical usage. Static exposure systems are limited by depletion of oxygen, accumulation of waste, and loss of test agent. They are generally unsuitable for most inhalation toxicology studies under current standards. Most modern inhalation exposure systems are of the dynamic type (Fig. 2). Dynamic exposure systems are characterized by a continuous replacement of chamber air and test material.

The concentration profile in a dynamic inhalation system rises rapidly, then asymptotically approaches a theoretical equilibrium value (Fig. 3). This phenomenon was reported by Silver (*12*), who also described the time necessary to reach a desired percent of the theoretical equilibrium concentration:

$$t_{99} = 4.605 \frac{\text{chamber volume}}{\text{chamber airflow}}$$

t_{99} = time to 99% of theoretical equilibrium concentration.

Referring to Figure 3, the exposure duration for a dynamic system is generally considered to be the interval between starting (ta) and stopping (tb) the generation system. The animals, however, remain in the exposure system for a time equivalent to the initial t_{99} (tc – tb). The suggestion by the National Toxicology Program (NTP) that exposure duration should be defined as the interval tc – ta has not met with universal acceptance (*4*).

The performance of inhalation exposure systems — i.e., leakage, material loss, and uniformity of concentration — has been addressed (*13,14*) and will not be reviewed here.

Table 1. Inhalation chamber environmental factors.[a]

Parameter	Effect
Temperature	Activity, ventilation
Relative humidity	Particle size, respiratory tract environment, ventilation
Atmospheric pressure	Ventilation cardiac function, respiratory tract environment
Airflow	Distribution, equilibration, contaminants
Air quality	Ventilation, stress
Noise/vibration	Stress

[a]From Phalen (*3*).

A requirement for currently performed inhalation studies is the ability to provide a consistently clean air supply, sourced from ambient air, to chambers housing control and treated groups. Environmental control, therefore, is an important aspect of inhalation exposure system design.

Unusual environmental conditions may place an additional, and unwanted stress on test animals. The ability to produce, control, and monitor the required environment should be a basic consideration in conducting inhalation studies. Environmental fact ors such as temperature, relative humidity, atmospheric pressure, air flow, air quality, noise, and vibration could affect the evaluation of toxicity (Table 1). Physical activity, respiratory patterns, and respiratory tract mucus may be affected by temperature and humidity. Not only is it important to have a well-controlled environment for a particular inhalation chamber, but all chambers used in a study should be controlled within the same limits. Variations in the inhalation chamber environment may affect experimental animals and exposure atmospheres (*15,16*).

One environmental parameter that has received particular attention is temperature. Temperature could have an important effect on survival of test animals. In a dynamic inhalation system, heat produced by test animals is transferred to chamber walls and then to exposure rooms by radiation, particularly in the case of steel walls. Heat from animals is transferred to the chamber air by convection (*16*).

Figure 4 presents a schematic of the inhalation chamber control system used at Lilly Research Laboratories. The system was designed to provide very clean air. It also controls, monitors, and reports the chamber's temperature, dew point, air flow and differential pressure, and room temperature. Recognizing that steel chamber walls provide little thermal insulation, the effect on chamber temperature related to the differential between chamber and room temperature was integrated into the control design. The system was designed to provide an initial room temperature 2°C below the chamber temperature set point. The system then adjusts room temperature so that chamber inlet duct heaters operate at 20 to 80% of capacity. Each parameter is controlled within user-defined limits and is accessible through a personal computer.

FIGURE 1. A series of 9 m^3 stainless steel and glass inhalation chambers (Lilly Research Laboratories).

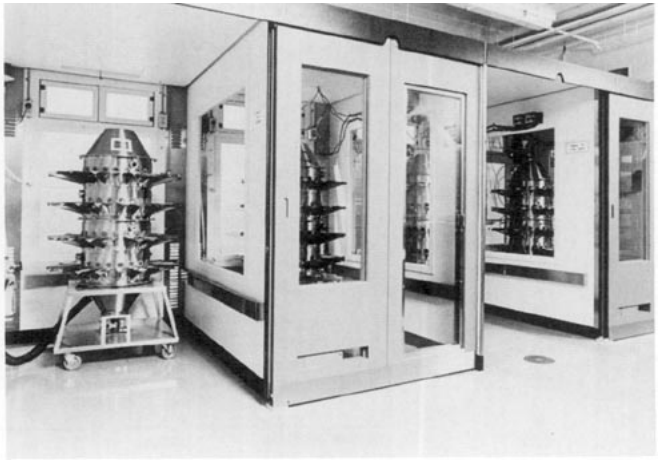

FIGURE 3. Concentration profile of a dynamic inhalation exposure system.

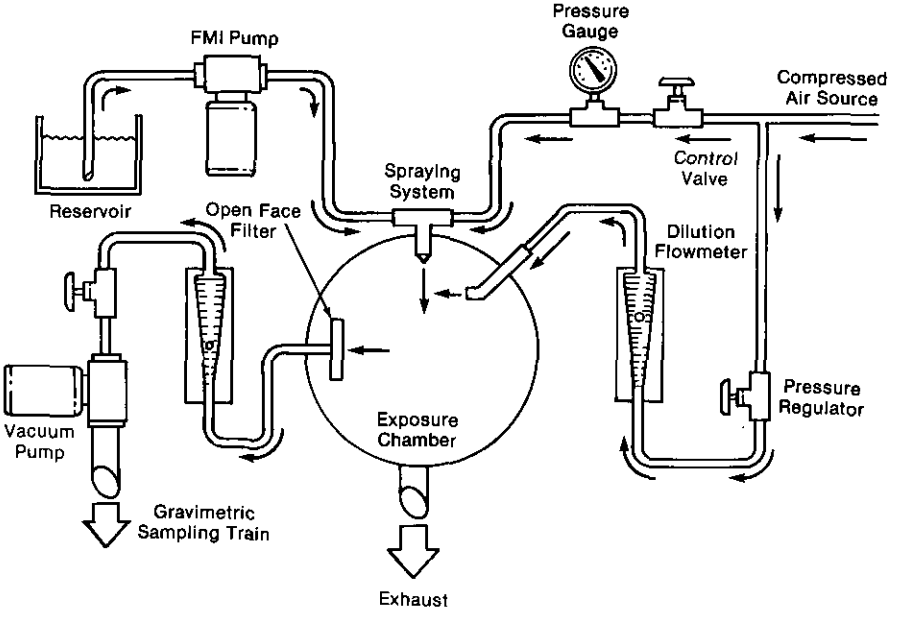

FIGURE 2. Dynamic inhalation exposure system.

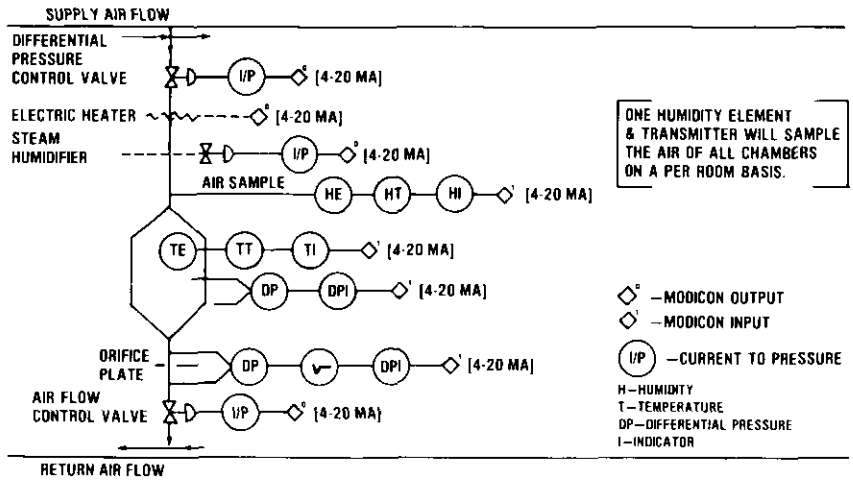

FIGURE 4. Schematic of environmental control system for inhalation exposure chambers. I/P, current to pressure; HE, HT, HI, dew point element, transmitter, and indicator, respectively; TE, TT, TI, temperature element, transmitter, and indicator, respectively; DP, DPI, differential pressure and differential pressure indicator; V, conversion of differential pressure to flow.

Table 2. The range of individual chamber conditions over a 90-day operation period.

Inhalation chamber number		Airflow, L/min[a]	Differential pressure, cm H_2O[b]	Temperature, °C[c]	Dew point, °C[d]
1	High	2007	2.03	21.0	11.3
	Low	1991	1.96	20.9	10.8
2	High	2005	2.02	21.0	11.4
	Low	1991	1.98	20.9	10.8
3	High	2008	2.02	21.0	11.4
	Low	1991	1.96	20.9	10.8
4	High	2006	2.03	21.0	11.1
	Low	1993	1.97	20.9	10.8

[a]Set point = 2000 L/min.
[b]Set point = 2.0 cm H_2O.
[c]Set point = 21°C.
[d]Set point = 11°C.

The system also allows for adequate control of multiple inhalation chamber environments (Table 2). Additional attention is given to chamber exhaust so that it is properly cleaned by baghouse and HEPA filters and vapor scrubbing systems.

As previously mentioned, whole body inhalation exposure systems have the disadvantage of multiple routes of exposure, i.e., dermal, ocular, oral, and inhalation. Griffis et al. (*17*) estimated 60 to 80% of dermally deposited material could reach the gastrointestinal tracts of some test animals.

Nose only exposure systems were designed to reduce or eliminate multiple routes of exposure (Figure 5). Most nose only systems, however, have not addressed the issue of providing fresh air and aerosol to animals in a vertical tier arrangement (Figure 6*A*). To address this, a multilevel flow-past chamber design (Figure 6*B*) was reported by Cannon et al. (*18*). The system apparently addresses the problems of large material requirements, surface losses, and aerosol depletion by test animals as one moves from the top tier to the bottom tier. In contrast to the conventional nose only exposure systems, each animal is supplied with fresh aerosol (Figure 6*A* and *B*).

As with whole body exposure systems, temperature is a critical parameter in nose only designs. Typically, each animal is restrained in glass, plastic, or steel restraining tubes (Figure 6). Since rats and mice regulate body temperature through their tails, a simple approach would be to allow their tails to protrude from the restraint tubes into a cooler ambient environment. Rats have been monitored for up to 8 hr in tail-out restraint tubes with no change in body temperature. An additional advantage of restraining tubes is the ability to monitor respiratory parameters during aerosol exposure (*19*).

Nose only or nose/mouth exposure systems are also applicable to studies with large animals. A typical system is designed around face masks (Figure 7). Poynter and Spurling (*20*) described a system for administration of metered dose inhalation (MDI) aerosols to test animals. Their system was modified to incorporate a flexible oropharyngeal tube and opposing one-way valves to limit rebreathing from the aerosol chamber (Figure 8). Inspiration and expiration are monitored, and MDI aerosols are shaken and delivered according to a predetermined sequence of breaths, only during inspiration.

The complete variety of aerosol generation techniques is beyond the scope of this overview.

Study Design

The design of inhalation toxicology studies should consider the need to evaluate both local and systemic effects of the test material and the formulation components. Acute inhalation toxicology studies are conducted with a single exposure of usually 1 to 4 hr (Table 3). The rat is the usual species for conducting acute inhalation studies, using either whole body or nose only exposure

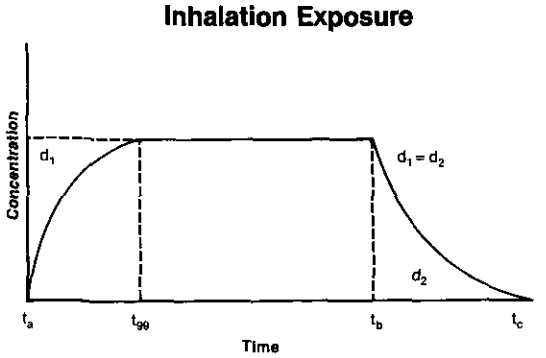

FIGURE 5. Nose-only exposure systems for rodents (Courtesy of BioResearch Laboratories, Quebec, Canada.)

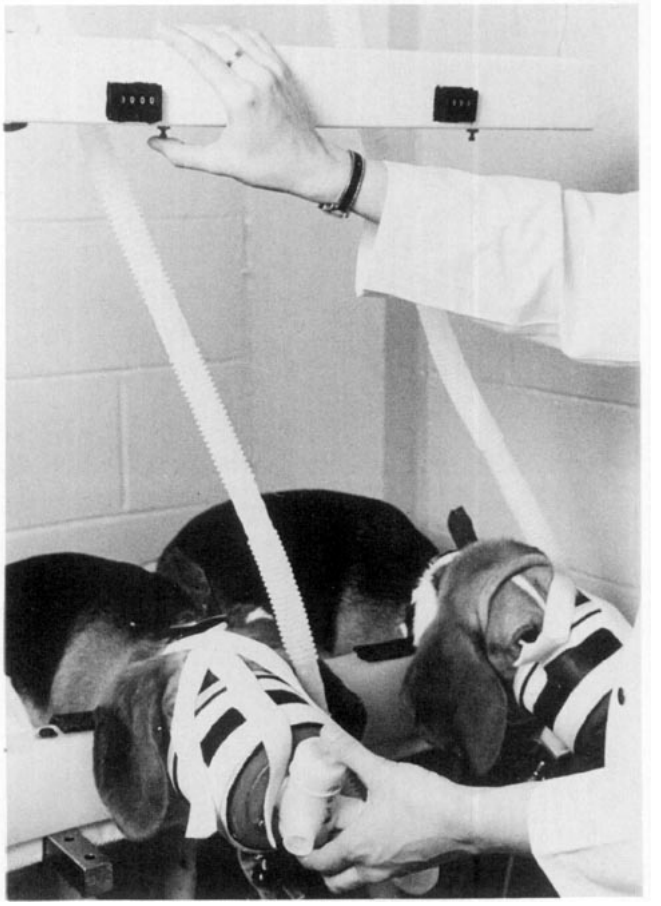

FIGURE 7. Mask-type exposure system for lavage animals. (Courtesy of BioResearch Laboratories, Quebec, Canada.)

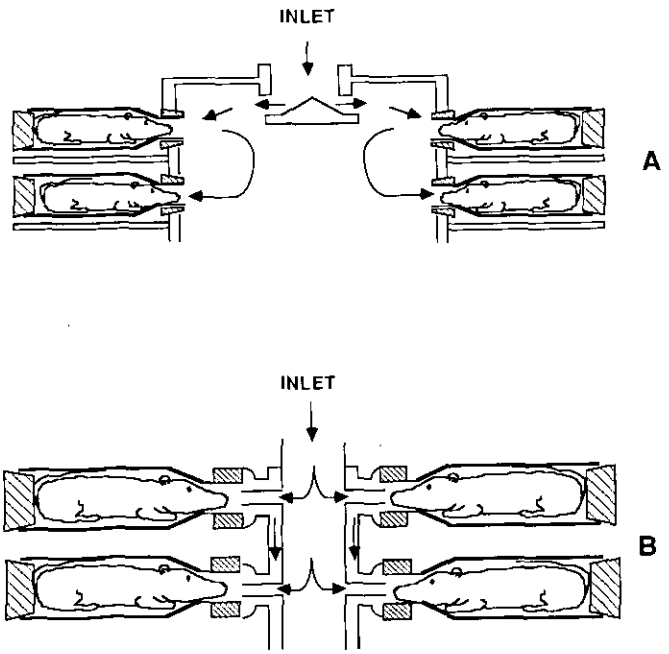

FIGURE 6. Diagram of nose-only inhalation exposure systems. (*A*) Conventional system; (*B*) flow-past system. Modified from Cannon et al. (*18*).

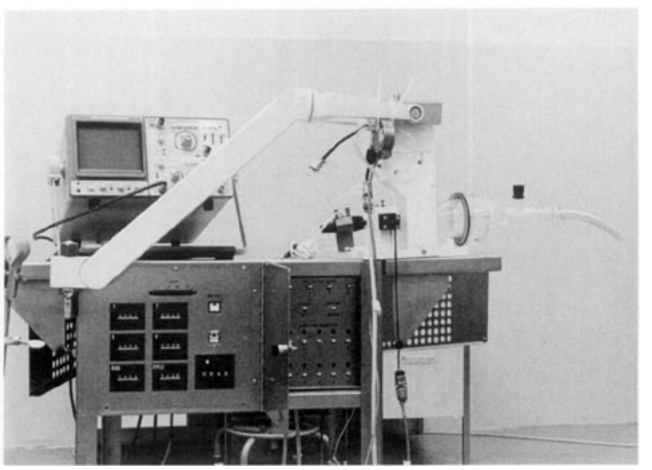

FIGURE 8. Metered dose inhaler dosing system (Lilly Research Laboratories).

Table 3. Acute inhalation toxicology study design.

Five animals/sex, two species
Up to five exposure levels
Whole body or nose/head only
Single 1- to 4-hr exposure
14-Day observation period
Necropsy (histopathology recommended)

Table 6. Subchronic inhalation toxicology study design.

10–30 animals/sex, plus satellite group, two species
Control plus at least three exposure groups
Whole body or nose/head-only
1–23 hr/day, 5–7 days/week, 52–104 weeks
Interim clinical evaluation
Necropsy/histopathology

systems. A 14-day observation period and a gross necropsy follow the single exposure. Histopathology is recommended but not required. The purpose of acute inhalation exposure studies is to establish an index of relative toxicity, i.e., lethality, and justify exposure concentrations for longer term studies.

Acute multidose inhalation studies (Table 4) are similar in design. Multiple exposures of 1 to 4 hr are conducted over a 14-day period, using either whole body or nose only exposure systems. Although control groups are not usually designed into single exposure acute studies, they are recommended for multiexposure studies. After the last exposure, a necropsy and histological evaluations are usually performed. The primary purpose is to choose exposure levels for subchronic studies.

Subchronic inhalation studies (Table 5) are conducted over a 30- to 180-day period. Exposure durations are usually 1 to 6 hr per day, 5 to 7 days per week. A control group and three to five exposure groups are usually used. Interim clinical evaluations, i.e., hematology, clinical chemistry, and urinalysis may be included. The exposure period is followed by a complete necropsy and histopathology. The primary purpose is to justify exposure concentrations for chronic studies. The data from all studies up to this point would be suitable for inclusion in investigational new drug applications or for setting preliminary worker exposure guidelines.

Chronic/oncogenic studies (Table 6) are conducted over the lifetime of the test species, usually rats and mice. The exposure duration may be up to 23 hr per day, 5 to 7 days per week, for 52 to 104 weeks. A complete necropsy and histopathology is performed after the last exposure. The purpose is to determine the toxicity due to extended repeat exposures, a no-observable effect level, and oncogenic potential. Gross (*21*) has prepared a review of regulatory guidelines and recommendations.

Table 4. Acute multiexposure inhalation toxicology design.

Five to ten animals/sex, two species
Control group plus up to five exposure concentrations
Whole body or nose/head-only
1–4 hr daily for 14 days
Daily observation
Necropsy/histopathology

Table 5. Subchronic inhalation toxicology study design.

10–20 animals/sex, two species
Control group plus three to five exposure concentrations
Whole body or nose/head-only
1–4 hr/day, 5–7 days/week from 90–180 days
Interim clinical evaluation
Necropsy/histopathology

The intranasal route is commonly used for peptide and protein drugs. Its advantages are: no metabolism in the gut wall, no destruction by gastrointestinal fluids, and avoidance of extensive first-pass hepatic metabolism. The intranasal route also provides an alternate to intramuscular administration. The exposure technology differs somewhat from conventional inhalation studies, but the study designs are similar. For all inhalation studies, gross examination of the nasal cavity at necropsy limits the usefulness of histopathologic examination. It is not recommended for studies specifically designed to determine effects on nasal mucosa (*22*).

Assessment of Toxicity

The assessment of toxicity in inhalation studies is similar to that used for toxicology studies conducted by any other route of administration. A range of doses/concentrations is studied; dose/concentration-related effects are evaluated, usually for both sexes, in one or more species.

Common protocols include the assessment of: body weight, food consumption, clinical signs of toxicity, clinical pathology (hematology, clinical chemistry, urinalysis), ophthalmology, gross necropsy (except nasal cavity), and histopathology. The purpose is to determine the inherent toxicity of specific agents. In doing so, one must make a distinction between toxicity and hazard (*21*). While many procedures are applicable to the assessment of inhalation toxicity, three that have been found useful in screening potential intranasal and pulmonary drugs will be briefly reviewed here.

Hussein et al. (*23*) described a method for studying nasal absorption and irritation in rats. Briefly, anesthetized rats are surgically prepared to allow retrograde perfusion of the nasal cavity while they are breathing through a tracheal cannula. This procedure was used to evaluate the bioavailability and nasal irritation of clofilium tosylate (*24*). Nasal administration of this drug appeared to be superior to oral administration, in terms of bioavailability. Dose-related nasal epithelial cell necrosis and sloughing were also reported. Even though surgical preparation was required, this procedure has proven useful in the screening of active ingredients, formulation acids, and complete formulations intended for intranasal administration.

Bronchoalveolar lavage from animals acutely exposed to various test materials, either by inhalation or instilla-

Table 7. Nasal cavity lavage (Swiss mice).

Test	LDH[a]	Protein[b]
SLS[c]	181	223
TX-100[d]	519	414
S85[e]	NR	NR
SCG[f]	NR	NR

[a] Percent of deionized water control: 16.7 ± 4.08 IU/L.
[b] Percent of deionized water control: 67.3 ± 29.94 μ/L.
[c] *SLS*, sodium lauryl sulfate.
[d] TX-100 = Triton X-100.
[e] S85 = Span 85.
[f] SCG = sodium chromoglycate.

tion, has been applied as a rapid screen for lung injury (*25,26*). The various lavage parameters that could be evaluated and their significance have been reviewed (*27*). In particular, total lactate dehydrogenase and its isoenzyme pattern can be used to detect respiratory tract toxicity. Beck et al. (*28*) suggested that lactate dehydrogenase levels in lung lavage fluid may not be appropriate for evaluating upper airway effects.

We have found that lavage of the upper respiratory tract, similar to the procedure described by Hussein et al. (*23*), did provide a measure of upper respiratory tract effects for sodium lauryl sulfate and Triton X-100. Span 85 and sodium chromoglycate did not produce a detectable response (Table 7). The usefulness of lavage fluid analysis is greatest as a comparative measurement of structurally and toxicologically related materials and somewhat less useful when comparing agents that act through different mechanisms (*28*).

Alarie (*29*) has written extensively on the sensory irritation technique. Briefly, mice are placed in a plethysmograph and exposed to increasing airborne concentrations of various agents. A concentration-related decrease in respiratory rate is found for those agents that stimulate this trigeminal reflex. Similar to the results of the lavage study mentioned above, sodium lauryl sulfate (Figure 9) and Triton X-100 were judged to be sensory irritants, while Span 85 and sodium chromoglycate were not.

When faced with the evaluation of many chemicals within a particular structure-activity relationship, the above methods rapidly provide comparative information in the form of acute and subacute responses.

Conclusion

Inhalation studies have been conducted for well over 100 years. While the basic principles may have changed little, inhalation technology has been in a state of continuous evolution. The need to develop techniques for testing a wide variety of gases, vapors, particulates, and droplets has led to the development of unique generating systems and the use of a variety of materials and designs for construction of inhalation chambers. Dose/concentration response relationships are usually evaluated using parameters common to most toxicology studies. Other procedures such as instillation, lavage, and airway irritation are useful for the rapid screening of agents for respiratory tract injury, especially when attempting to discriminate between related members of a class of drugs under development.

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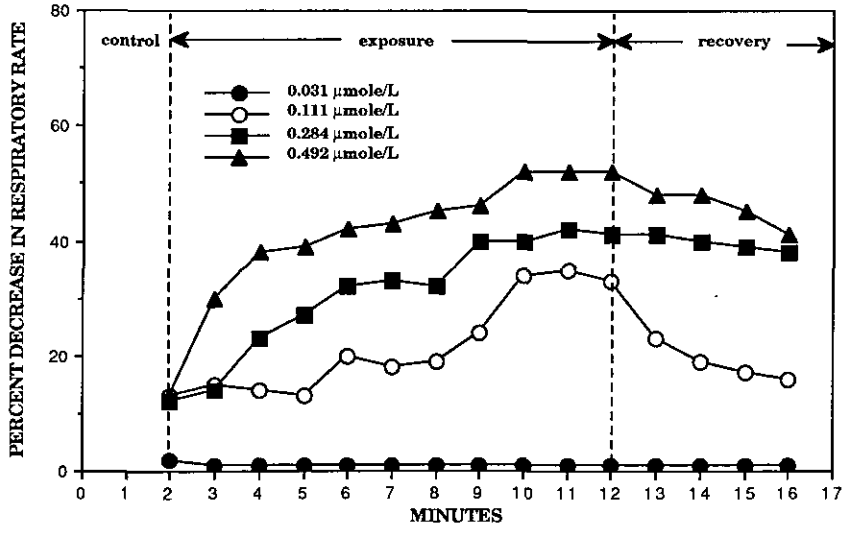

FIGURE 9. Sensory irritation response of sodium lauryl sulfate.

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